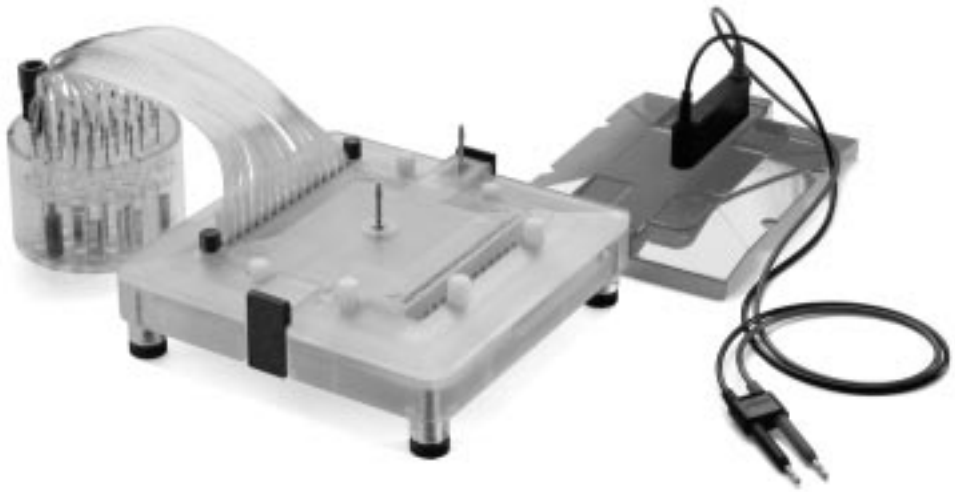


Whole Gel Eluter
and
Mini Whole Gel Eluter

Instruction Manual

Catalog Numbers
165-1250
165-1251
165-1255
165-1256

BIO-RAD

Table of Contents

Section 1
General Information

1.1 Introduction

The Whole Gel Eluter provides an alternative method for recovering samples from SDS and Native PAGE slab gels. It is a preparative electrophoresis instrument which allows simultaneous electro-elution of multiple protein bands separated on a polyacrylamide gel. Elution is in the transverse direction, through the thickness of the gel. Proteins are eluted into narrow chambers with each of these fractions containing individual or multiple protein bands.

The eluter consists of a solid acrylic base, a drop-in bottom electrode, an elution chamber core, an upper plate/spring electrode and a lid. The Whole Gel Eluter comes in two sizes. The large format accommodates slab gels up to 20 cm long and the small format is designed for mini-gels (Mini-PROTEAN® II and Ready Gels). Filter paper and a cellophane sheet are placed between the bottom electrode and the elution chamber core. The gel is sandwiched between the top of the elution chamber core and the spring electrode. During elution, the proteins are captured in one of the 30 elution chambers of the large eluter or one of the 14 chambers of the mini eluter.

One important application for this instrument is the purification and subsequent direct screening of protein mixtures. Andersen and Heron describe a direct cellular analysis of a complex protein mixture for biological activity.[1] The eluter acts as an electrodialyzer removing SDS from protein, leaving them in a non-toxic physiological buffer that can then be used directly in a cellular assay. The Rotofor® and Prep cell are recommended for larger scale electrophoretic protein purification.

Simple procedures are provided in this manual for optimizing elution conditions. It is recommended that these procedures be performed for each new sample and when making changes to slab gel electrophoresis conditions.

1.2 Specifications

Construction

Base	acrylic/polycarbonate insert
Elution chamber core	acrylic/polycarbonate insert
Bottom electrode	platinum coated titanium plate
Upper plate/spring electrode	acrylic/platinum coated titanium plate
Lid	polycarbonate
Cutting template	acrylic

Consumables	**Large**	**Mini**
Upper filter paper	50 sheets	50 sheets
Lower filter paper	75 sheets	50 sheets
Sealing tabs	50 tabs	50 tabs
Cellophane membrane	25 sheets	25 sheets

Other Specifications	**Large**	**Mini**
Shipping weight	9.25 lb	4.75 lb
Overall size	5.0” H x 6.0” W x 6.0” D	5.0” H x 10.5” W x 10.5” D
Voltage limit	300 volts	200 volts
Power limit	15 watts	10 watts
Elution sample volume	~3 ml	~0.5 ml

1.3 Safety

Power to the Whole Gel Eluter is to be supplied by an external DC power supply. This power supply must be ground isolated in such a way that its DC voltage output floats with respect to ground. The recommended power supply for this instrument is the PowerPac 300. The maximum specified operating parameters are:

Whole Gel Eluter	**Mini-Whole Gel Eluter**
300 VDC	200 VDC
15 W	10 W

The lid assembly provides a safety interlock for the unit. Current flow to the cell is broken when the lid assembly is removed. Do not attempt to circumvent this safety interlock and always turn off and disconnect the power supply when working with the cell.

The recommended power supplies are ground isolated by design to minimize potential shock hazard. However, working around high voltage equipment in a laboratory environment is potentially dangerous. It is the user's responsibility to always exercise care in setting up and running electrophoresis instruments. Always turn off the power supply before removing the lid. Do not attempt to use the cell without the safety lid.

Important:

This Bio-Rad product is designed and certified to meet EN61010-1 safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will:

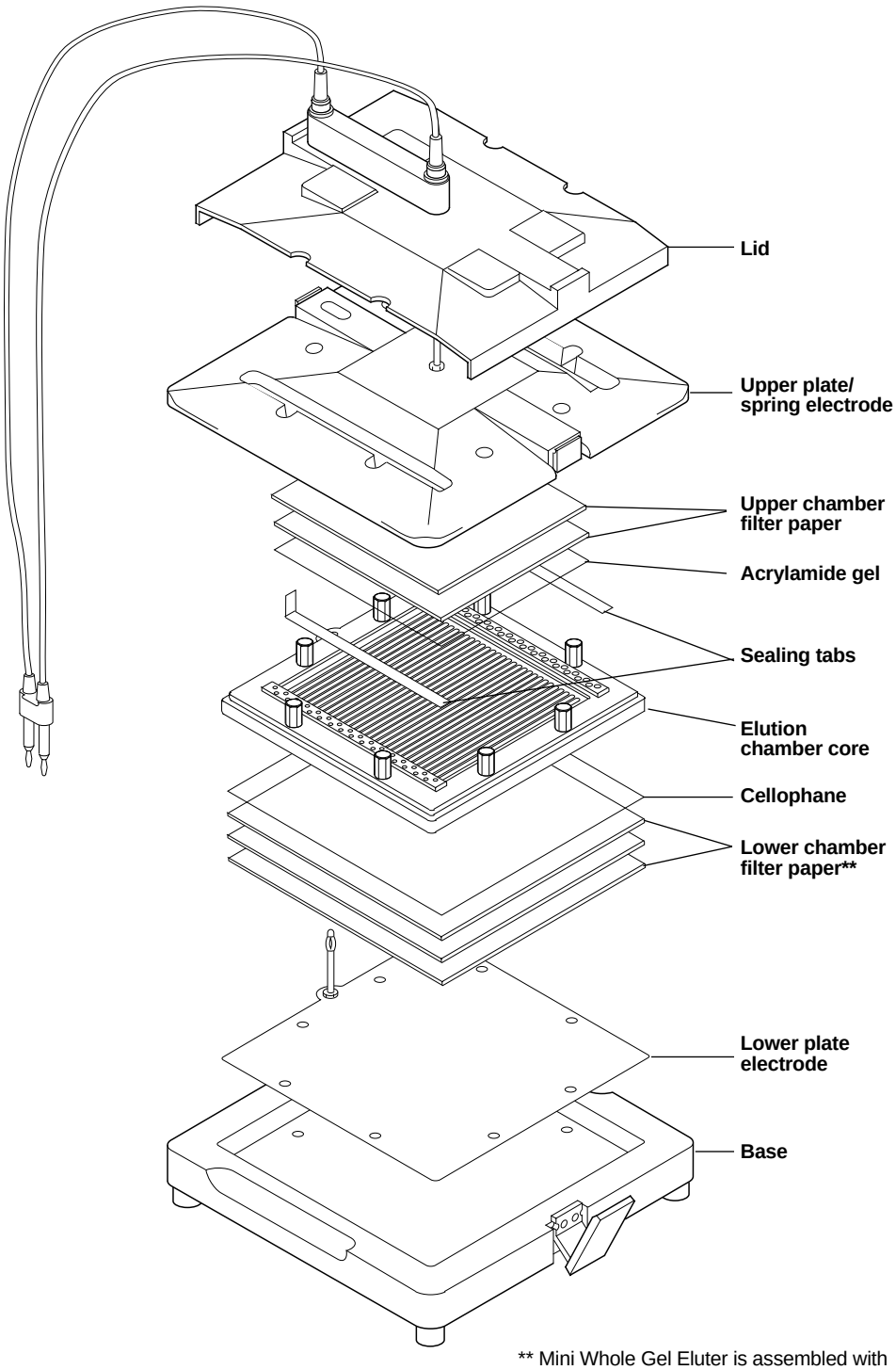
- Void the manufacturer's warranty
- Void the EN61010-1 certification, and
- Create a potential safety hazard

Bio-Rad is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

This product conforms to the class A standards for Electromagnetic Emissions, intended for laboratory equipment applications. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or on the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

* EN61010-1 is an internationally accepted electrical safety standard for laboratory instruments.

Section 2
Description and Assembly of Components



** Mini Whole Gel Eluter is assembled with 2 sheets of lower chamber filter paper.

Whole Gel Eluter exploded view.

2.1 Whole Gel Eluter Components

Base and Lower Electrode

The base forms a stable foundation for the eluter. The recess in it houses the lower electrode, the lower filter paper, the cellophane membrane and the elution chamber core. Adjustable feet enable leveling of the eluter.

Elution Chamber Core

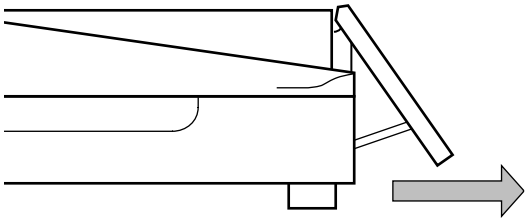
The elution chamber core is a multi-channel collection vessel which is dropped into the base plate and screwed down over the filter paper and cellophane membrane. Proper sealing of the core is important for preventing cross-contamination of the fractions. Proteins eluted from the gel are held in the individual chambers until they are collected with the vacuum harvester or transfer pipet.

Upper Plate/Spring Electrode

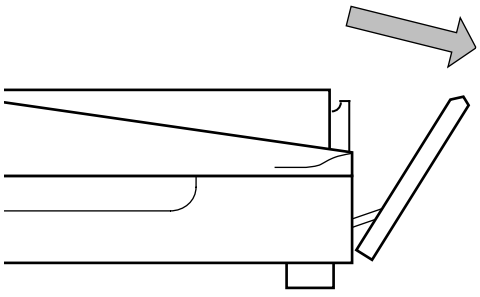
Attached to the upper plate is the spring electrode. The electrode makes contact with the upper filter paper. The tension on the spring holds the slab gel in place and seals the tops of the elution chambers.

Clamps

The upper plate and base plate are held together by clamps on the sides of the eluter. To open the clamps of the Whole Gel Eluter, pull the bottom outward as shown in Figure A. Then disengage the clamp as shown in Figure B. **The Mini-Whole Gel Eluter has its clamps mounted in the opposite direction.** To open the clamps of the Mini-Whole Gel Eluter, pull the top outward. Then disengage the clamp at the bottom. To close the clamps, reverse these procedures.



A. Step 1



B. Step 2

Opening Whole Gel Eluter Clamps

Lid

The lid covers the electrical contacts during elution to provide electrical insulation. The lid cannot be removed without breaking the electrical circuit. It can be placed on the eluter in only one alignment, so that the anode and cathode connections cannot be accidentally reversed.

Cutting Template

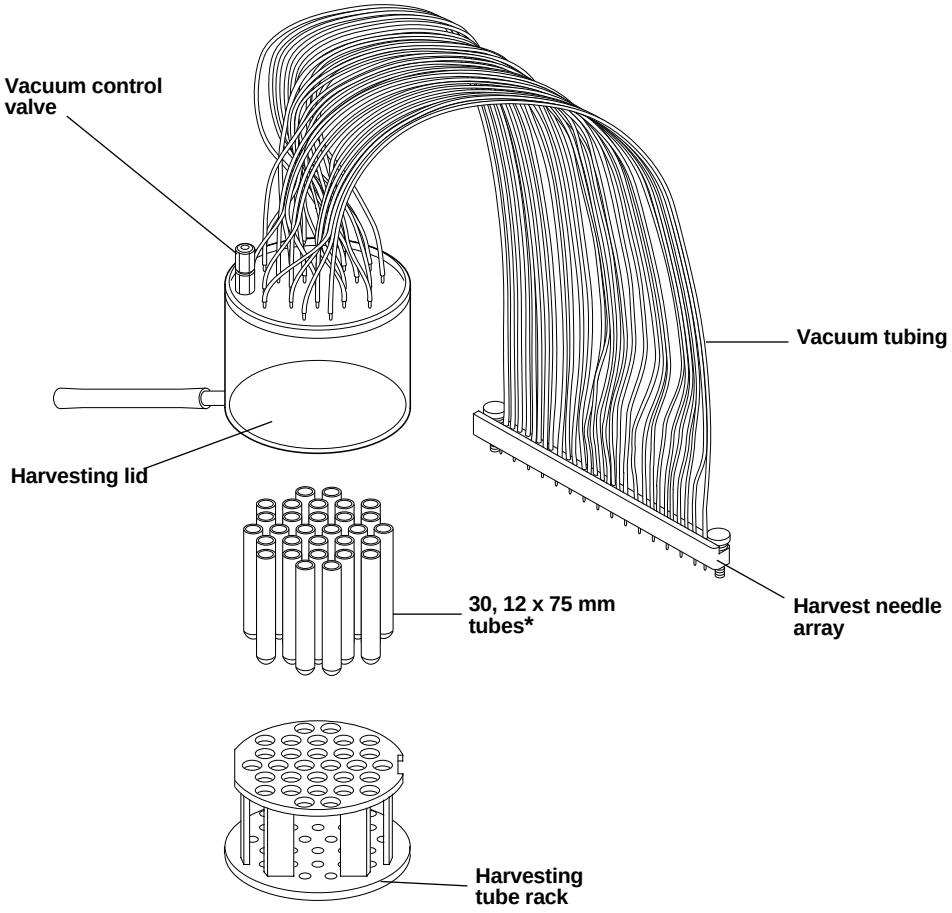
The cutting template is a guide for excising the portion of the slab gel intended to be eluted. It also functions as a template for cutting your own upper and lower filter paper and cellophane membrane.

Ruler

The ruler has been calibrated to the distance between elution channels. It can be used as a guide to approximate where individually resolved protein bands may elute.

In order to purify an individual protein, the nearest contaminant must be at least 5 mm away on a preparative gel for the Mini Whole Gel Eluter or 6 mm for the Whole Gel Eluter.

2.2 Vacuum Harvester Components



* The Mini Whole Gel Eluter uses 14, 1.5 ml microcentrifuge tubes.

Tube Rack

The tube rack forms a stable base for the harvester as well a housing for the collection tubes. The rack is numbered for easy identification of fractions.

Lid and Needle Array

The lid fits onto the base only one way for proper alignment of the harvester needles with the collection tubes. Connected to the lid by tubing is the needle array. The needle array inserts into the harvest ports on the left side of the eluter. Thumb screws hold the needle array in place, forming a seal between the array and the eluter. The array is designed to be inserted into the eluter collection ports only one way for proper alignment during harvesting.

Vacuum Control Valve

The vacuum control valve is attached to the lid of the harvesting unit. The valve can be gradually adjusted to increase or decrease the vacuum preventing uneven harvesting and splattering of eluates.

Section 3
Assembly and Operation

3.1 Preparative Slab Gel Electrophoresis

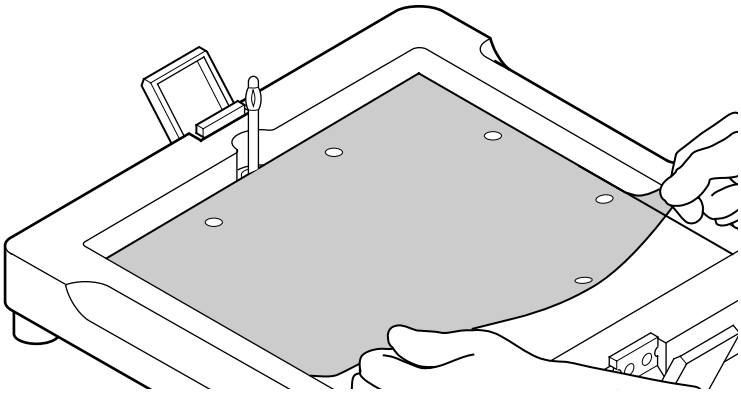
The Whole Gel Eluters can accommodate SDS or Native PAGE preparative gels up to 3 mm thick. Gel thickness can have a major effect on the elution and recovery efficiency of proteins. In addition, proteins not eluted into a buffer containing SDS, may adhere to the cellophane (or dialysis) membrane. The thicker the gel, the longer it will take to elute the proteins and the longer they will be in contact with the membrane. Protein recovery can be greatly improved by reversing the current for 10 to 15 seconds after elution and by keeping elution times down to a minimum.

1. Run the SDS or Native gel with a preparative comb following standard electrophoresis procedures. Refer to Sections 6–7 and References for information on running SDS and Native PAGE gels. The Mini Whole Gel Eluter requires a preparative gel with a minimal width of 6.5 cm and the large format eluter requires a minimal width of 14.5 cm. The length of the gel is not critical. It is acceptable for some unused elution chambers to be left empty. It is convenient to have a lane of prestained standard proteins adjacent to the preparative well to facilitate alignment of the cutting template, but this lane must be cut off before placing the gel in the eluter.
2. Make up a liter of elution buffer for the Whole Gel Eluter and 500 ml for the Mini Whole Gel Eluter. This buffer will be used to equilibrate the preparative slab gel and for the subsequent eluter assembly. Refer to Sections 4.1 and 7.4 for elution buffer selection.
3. Following electrophoresis, remove the gel from the gel cassette and equilibrate it in elution buffer for 15–20 minutes. This is to allow an exchange of buffers and minimize swelling of the gels during elution.

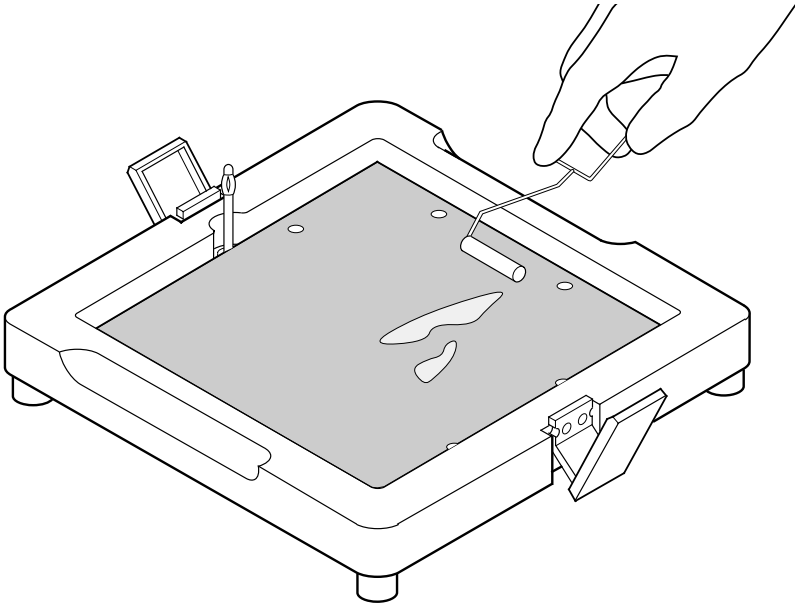
3.2 Whole Gel and Mini Whole Gel Eluter Assembly

One of the most important factors in the successful use of the Whole Gel Eluter is the assembly of the unit. As with blotting techniques, it is vital to remove and (or) prevent bubble formation under the gel. Second, proper alignment of the protein bands parallel to the elution chambers enhances selectivity.

1. Place the base of the eluter on a level surface. Place the leveling bubble in the center of the base and use the adjustable feet to level the base. Insert the bottom electrode plate in the depression in the base. Align the electrode with the holes for the screws and electrode stem.
2. Moisten two or three sheets of precut lower chamber filter paper with elution buffer and lay them on top of the bottom electrode plate. The mini eluter requires two sheets and the large eluter requires three sheets of filter paper to seal the bottoms of the elution chambers.
3. Wet a precut sheet of cellophane (or dialysis) membrane and place it on the blotting paper. Work out all air bubbles with the roller provided.

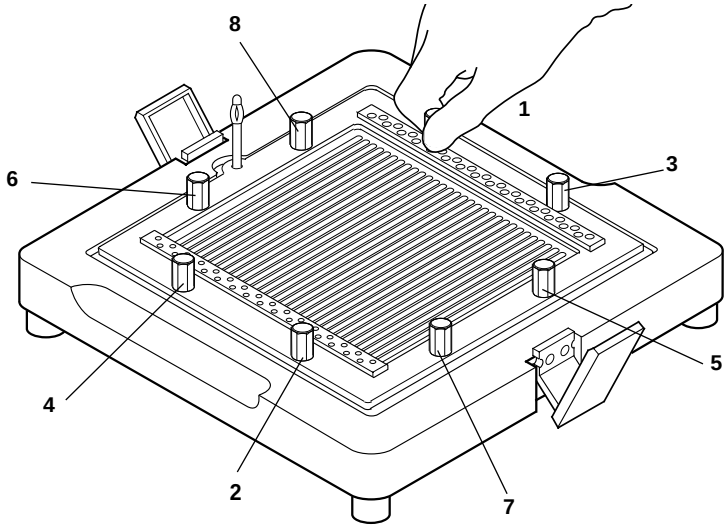


Wet a precut sheet of cellophane and place it on the precut, wetted filter paper.



Using the roller, work out all air bubbles.

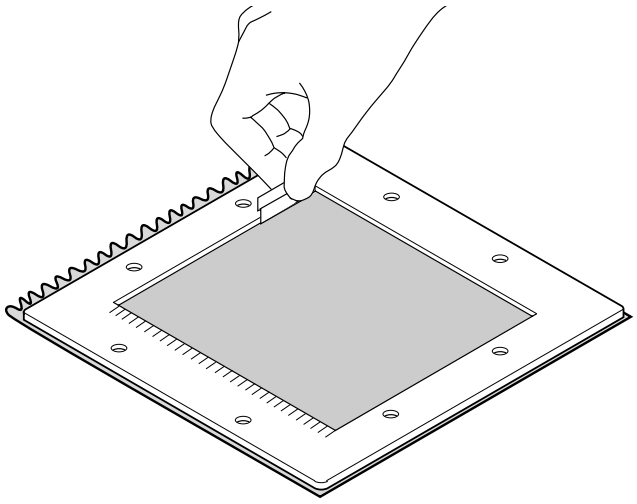
4. Insert the elution chamber core, aligning the holes for the screws and the electrode stem. **Finger tighten the hex screws in numerical order (crisscross pattern) while pushing down on the core.** (See diagram below.)



Finger tighten the hex screws in numerical order (criss-cross pattern) while pushing down on the core.

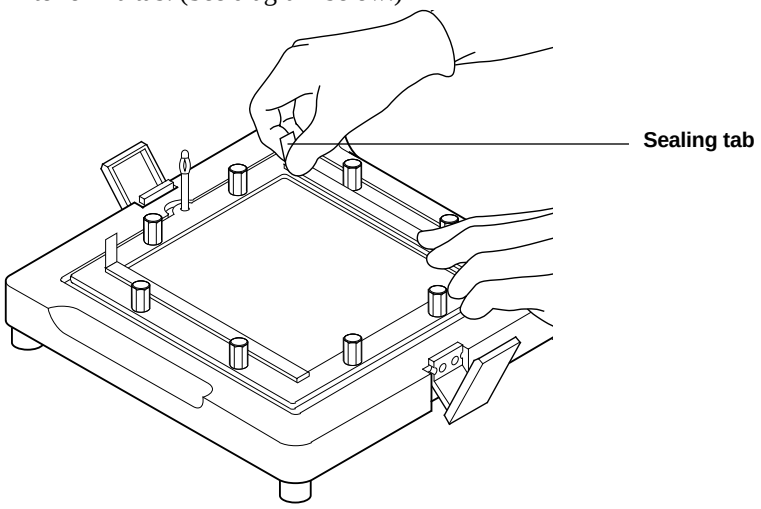
5. Turn the assembly upside down over a sink and shake out any excess buffer squeezed into the buffer overflow channel during the tightening of the screws.
6. Fill the elution chamber with elution buffer so as to cover all of the channels. Carefully remove any bubbles trapped in or around the channels.
7. Lay the preparative gel on a clean flat surface and place the cutting template on top of it. Use the template to outline the portion of the gel to be eluted. The template is made of clear acrylic and has numbered lines with which to align the template with the dye front and molecular markers. Wear clean gloves when handling the gel.

8. Carefully excise the part of the gel within the template with a razor. (See diagram below.) **Use a downward, chopping motion when cutting the gel.** Drawing the blade through the gel will cause it to tear. It is very important to insure parallel alignment of the protein bands with the chambers of the eluter for sharply defined and reproducible fractionation of proteins. With experience, it should be possible to achieve a high degree of gel-to-gel reproducibility.



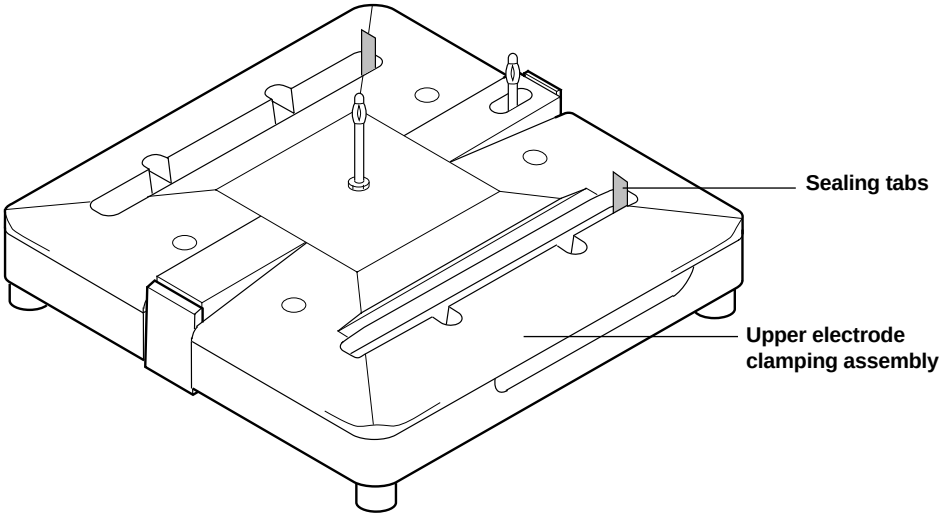
Carefully excise the part of the gel within the template with a razor.

9. Starting at one end, gently lay the excised gel in the elution chamber so that the protein bands are parallel to the channels. Do not introduce air bubbles under the gel.
10. Soak two sheets of precut upper chamber filter paper in the elution buffer, then lay them on top of the gel, working out any air bubbles with the roller provided.
11. Blot any excess buffer and thoroughly dry the two raised areas containing the aspiration ports. Seal these ports with the tabs provided or use transparent tape folded back on itself to form a tab. (See diagram below.)



Sealing tabs cover all elution ports before running the eluter.

12. Thread the tabs through the cutouts of the upper electrode/clamping assembly. (See diagram below.) Place the upper electrode assembly on top of the chamber, aligning the holes for the screw heads and the stem of the bottom electrode. Gently push down while closing the side clamps.



Confirm that the sealing tabs are threaded through the cutouts of the upper electrode/clamping assembly.

3.3 Running the Whole Gel Eluter

1. Place the lid on top of the assembled eluter so that it is fully enclosed. Note that the lid can be placed in only one orientation, so that the anode and cathode connections cannot be reversed.
2. Attach the electrical leads to a suitable power supply with the proper polarity.
3. Apply power to the Whole Gel Eluter to begin the elution. As a safety precaution, do not exceed the voltage and power limits. See Section 4.2 for specific running conditions.
4. Turn off the power at the end of the elution time. Reverse the polarity of the electrical leads at the power supply. Apply reverse current to the Whole Gel Eluter for 10–15 seconds to dislodge proteins bound to the cellophane (or dialysis) membrane.

3.4 Harvesting Eluate

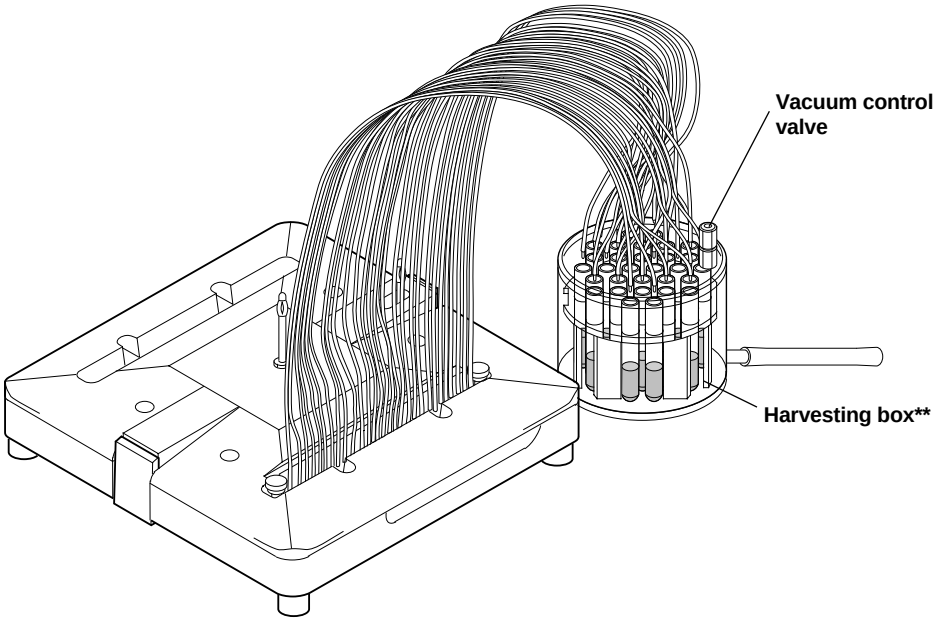
Vacuum Harvesting

1. After elution is complete, turn off the power supply, disconnect the electrical leads and remove the lid. **Do not disassemble the eluter.**
2. Remove the harvest port sealing tabs from both sides of the eluter. Grasp the tabs and gently pull them away from the eluter. Pulling too quickly will disturb the samples. Some spraying from the neighboring holes is normal and not caused by too much elution buffer.
3. For the large format harvester, load the rack with thirty 12 x 75 mm test tubes. Use fourteen 1.5 ml microcentrifuge tubes with separate press on or screw on lids for the mini-format harvester. Tubes with caps attached to them will not fit in the rack.

4. Place the lid on top of the harvester. Note that the lid is keyed to the base for proper alignment of harvester needles and collection tubes.

5. Attach the harvester to a house vacuum or other vacuum source in the off position. Insert the needle array into the harvest ports on the left hand side of the Whole Gel Eluter (unnumbered side). Tighten the thumb screws on both sides of the needle array so that the gasket seals against the harvest ports. Note that the needle array is keyed for proper alignment.

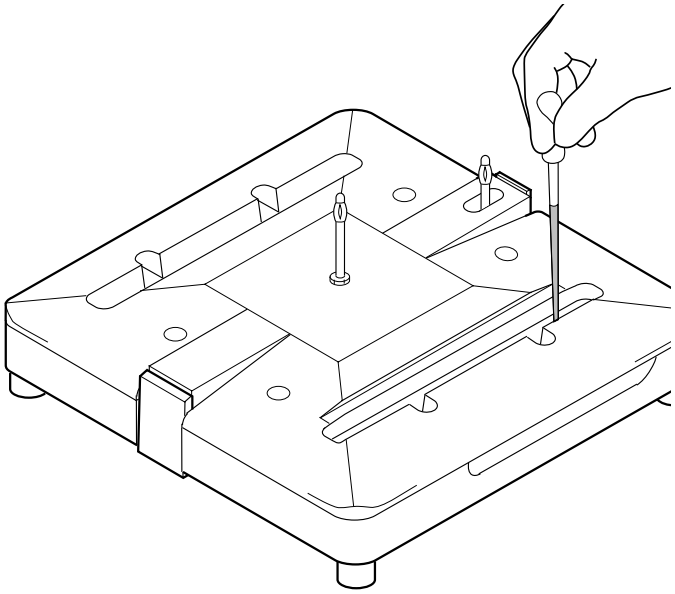
6. With the vacuum control valve fully open, turn on the vacuum source. Slowly close the control valve to draw a gentle vacuum with which to aspirate the eluates. **Aspiration should be done slowly to prevent splattering and uneven harvesting.** It may be necessary to slightly tilt the eluter toward the collection ports to facilitate complete aspiration of the eluates.



** Thirty tubes of eluate from the Whole Gel Eluter are collected in the Harvesting Box and fourteen tubes are collected from the Mini-Whole Gel Eluter.

Manual Harvesting

1. If a vacuum harvester is not available, eluates may be removed with the proper size Bio-Rad Disposable Plastic Transfer Pipet (DPTP). The numbered harvest ports on the right side of the eluter are used for manual aspiration of samples. The mini format eluter requires Bio-Rad DPTP style M (catalog number 223-9563) and the large format eluter requires Bio-Rad DPTP style D (catalog number 223-9523).



Eluates are collected manually with a Disposable Plastic Transfer Pipet (DPTP).

Section 4
Optimizing Conditions

4.1 Buffer Selection

SDS-PAGE or Native PAGE preparative gels can be eluted on the Whole Gel Eluter using denaturing (SDS) or non-denaturing elution buffer systems. Virtually any buffer can be used if it proves appropriate for the proteins under investigation, which must be determined empirically. **Buffers used for elution are not limited to those described in the following sections.**

SDS-PAGE

Preparative SDS-PAGE gels can be eluted with either a denaturing or non-denaturing buffer. The advantage of using a non-denaturing buffer is that the electro-elution process strips much of the SDS off the proteins, leaving proteins in a non-toxic buffer that can be used directly in a cellular assay. See the Table in Section 7.4 for a list of non-denaturing elution buffers. The addition of SDS to the elution buffers has the advantage of preventing proteins from adhering to the cellophane (or dialysis) membrane and insuring complete electro-elution of all proteins.

Native PAGE

Preparative Native PAGE gels are usually eluted with non-denaturing buffer systems (see Section 7.4 for buffers useful in non-denaturing elution). The buffer initially used in running the preparative Native PAGE gel should be the first non-denaturing buffer tried for elution.

In contrast to SDS-PAGE where proteins migrate according to size only, the mobilities of proteins in native PAGE systems depends on both their charges and sizes. There is no single elution/electrophoresis buffer system that will optimally elute all native proteins. When selecting conditions for the elution of native proteins it is important to consider the pI of the protein under investigation relative to the pH of the buffer system.

The most important consideration for optimum elution/electrophoresis of a protein is the pH of the buffer. The pH of the buffer must be within the pH range over which the proteins under study are stable and retain biological activity. In addition, the pH of the chosen buffer system must impart sufficient charge to the protein for it to move through the gel at a reasonable rate during the run.

The pH of the elution buffer determines the charges (and shapes) of proteins. Therefore, during native elution/electrophoresis, the pH of the buffer will affect the migration of the proteins. For example, a buffer with an alkaline pH value relative to the pI of a particular protein will impart net negative charge to the protein. In such a buffer system, the protein migrates toward the positive electrode (anode). Elution buffers with acidic pH values relative to the pI of a protein impart net positive charge to the protein so that it migrates toward the negative electrode (cathode). A buffer with a pH value identical to the pI of a protein results in net neutral charge on the protein and it will not move at all in an electric field. Buffers with pH values far from the pI of the proteins result in fast elution/electrophoresis rates.

4.2 Running Conditions

The McLellan buffers described in this manual are a good starting point when selecting an elution buffer (Section 7.4).[2] Eluting with any one of these buffers will give similar voltage and current readings based on their similar ionic strengths.

The elution rate of any given protein will vary depending on its pI and the pH of the elution buffer. As a general guide the buffer should be at least two pH units away from the pI of the protein to impart sufficient charge to the protein. This must be tempered when the stability and biological activity of the protein must be maintained by a given pH range. Proteins bound with SDS will have a net negative charge. Under these conditions elution is best in a high pH buffer. Determining the proper elution time will require following the optimization procedure detailed below (Section 4.3).

Elution Buffers	Constant Current	Elution Time
Large Eluter	200–250 mA	10–30 min
Small Eluter	75–100 mA	10–30 min

Note: Elution parameters are based on use of the McLellan buffers in the pH range of 8.1–10.2 to elute *E. Coli* lysate from a 1 mm thick 12% SDS-PAGE gel. A total of 200 µg of protein was loaded per gel. Elution times will vary with buffer pH, protein pI, gel thickness, and gel porosity.

4.3 Optimization Procedure

There are multiple factors that can affect the elution of a protein or mixture of proteins from a slab gel (*i.e.* protein pI, buffer pH, molecular weight, gel porosity, gel thickness, etc.). Therefore, for every new sample, change in elution buffer or running conditions, and when making changes to the conditions of the initial preparative slab gel electrophoresis, we recommend doing the following optimization procedure.

1. Run a preparative gel with your sample. Make sure that the parameters used for this gel are the same ones used for subsequent elutions (*i.e.* denatured or non-denatured gels and gel thickness).
2. Follow Sections 3.1–3.2 for the setup of the Whole Gel Eluter, except excise the preparative gel so that the separated protein bands run perpendicular to the elution chambers and not parallel to them.
3. Run the Whole Gel Eluter according to Section 3.3.
4. Eluted samples are harvested over a time course. On the first time point, turn off the power supply and harvest one elution chamber with a disposable transfer pipet. Re-establish the current to the eluter after sampling.
5. Repeat step 4 for each time point until all of the chambers have been harvested. Then assay the samples or total protein.
6. Plotting protein concentration versus sampling time will show the optimum elution time for the buffer system and set running conditions.

Following every elution run, stain the gel and the membrane with Coomassie® Blue. This will indicate the completeness of the elution and whether proteins were lost due to membrane binding.[1]

Section 5
Maintenance and Sterilization

5.1 Cleaning the Whole Gel Eluter

The Whole Gel Eluter should be cleaned after every use. The base plate, bottom electrode, elution chamber core and upper plate/electrode should be washed with a laboratory detergent (for example, Bio-Rad Cleaning Concentrate, catalog number 161-0722), then rinsed thoroughly with distilled water. The lid with electrical leads should be wiped with a damp cloth and allowed to dry thoroughly before use.

5.2 Sterilization of the Whole Gel Eluter

The Whole Gel Eluter is not autoclavable. The unit may be sterilized with 70% ethanol. Do not attempt to sterilize the lid (ethanol will cause crazing of the plastics). The blotting paper and cellophane (or dialysis) membrane may be autoclaved if desired.

5.3 Cleaning the Vacuum Harvester

The harvester and tube rack should be washed with a laboratory detergent, then rinsed thoroughly with water. The vacuum tubing and needle array should be thoroughly rinsed as well. Turn the harvester lid upside down and fill it with water. This will allow water to flow through the tubing and out the array. Dry the tubing by pulling air through the harvester while it is attached to a vacuum source.

Note: Do not attempt to sterilize the Vacuum Harvester. Use autoclaved or pre-sterilized transfer pipets if sterile harvesting is required.

Section 6
SDS-PAGE

6.1 Reagents for SDS-PAGE Slab Gels (Laemmli buffer system)[3]

Premixed liquid acrylamide (concentrated solution) and premixed electrophoresis buffers are available (see product information section). Sections 6.2–6.4 describe preparation of polyacrylamide gels using the following stock solutions.

A. 30% Acrylamide Stock Solution

Acrylamide/Bis (30% T, 2.67% C)

146 g acrylamide (29.2 g/100 ml)

4 g N'N'-Bis-methylene-acrylamide (0.8 g/100 ml)

Dissolve in about 350 ml deionized water.

Make to 500 ml with distilled water. Filter and store at 4 °C in the dark.

Or substitute Bio-Rad's Preweighed Acrylamide/Bis 37.5:1 mixture or 40% Acrylamide/Bis stock solution.

Caution: Acrylamide monomer is a neurotoxin. Avoid breathing acrylamide dust, do not pipet acrylamide solutions by mouth, and wear gloves when handling acrylamide powder or solutions containing it. For disposal of unused acrylamide, add bis-acrylamide (if none is present), induce polymerization, and discard the solidified gel.

B. Separating (resolving) Gel Buffer Stock

1.5 M Tris-HCl, pH 8.8

Dissolve 54.5 g Tris base in approximately 150 ml deionized water.

Adjust to pH 8.8 with 1 N HCl (Do not back titrate).

Make to 300 ml with deionized water and store at 4 °C.

C. Stacking Gel Buffer Stock

0.5 M Tris-HCl, pH 6.8

Dissolve 6 g Tris base in approximately 60 ml deionized water.

Adjust to pH 6.8 with 1 N HCl (Do not back titrate).

Make to 100 ml with deionized water and store at 4 °C.

D. Sample Buffer (SDS-reducing buffer)

Deionized water	2.8 ml
0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	2.0 ml
10% (w/v) SDS*	1.6 ml
ß-mercaptoethanol	0.4 ml
0.5% (w/v) bromophenol blue	0.2 ml
Total Volume	8.0 ml

Mix equal volumes of sample buffer and sample, and heat at 95 °C for 4 minutes. SDS reducing buffer is 0.06 M Tris-HCl, pH 6.8, 2% SDS, 5% beta-mercaptoethanol, 25% glycerol (w/v) and 0.01% bromophenol blue.

*To make a 10% SDS solution: Dissolve 1.0 gram of SDS in deionized water with gentle stirring, then adjust the volume to 10 ml with water.

E. 10x Electrode (Running) Buffer, pH 8.3 (Makes 1 Liter)

Tris base	30.3 g
Glycine	144.0 g
SDS	10.0 g

Dissolve and adjust to 1,000 ml with deionized water. **DO NOT** adjust pH with acid or base. To make 1 liter of 1x electrophoresis buffer (0.025 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3) dilute 100 ml of 10x stock with 900 ml of deionized water.

6.2 Preparing SDS-PAGE Gels of Any %T

The following table can be used to prepare Tris-HCl acrylamide gels from 4% to 17 % T. Sections 6.3 and 6.4 provide detailed formulas for calculating specific gel types. Acrylamide/Bis stock solution of 30% (37.5:1) is used. The amounts listed for the components in the table below are based on a total volume of 10 ml. When SDS is included in the sample buffer and the upper electrophoresis running buffer it can be left out of the gels during their preparation.

% T (acrylamide monomer)	Deionized H_2O (ml)	Gel buffer solution* (ml)	Acrylamide/Bis solution 30% stock (37.5:1) (ml)
4%	6.15	2.50	1.33
5%	5.80	2.50	1.67
6%	5.55	2.50	2.00
7%	5.15	2.50	2.33
8%	4.80	2.50	2.67
9%	4.47	2.50	3.00
10%	4.17	2.50	3.33
11%	3.80	2.50	3.67
12%	3.47	2.50	4.00
13%	3.15	2.50	4.33
14%	2.80	2.50	4.67
15%	2.47	2.50	5.00
16%	2.15	2.50	5.33
17%	1.80	2.50	5.67

* Resolving Gel buffer - 1.5 M Tris-HCl, pH 8.8

* Stacking Gel buffer - 0.5 M Tris-HCl, pH 6.8

Catalysts	10% APS*	TEMED*
Resolving Gel	50 µl	5 µl
Stacking Gel	50 µl	10 µl

* Amounts are per 10 ml gel volume. To make 10% APS, dissolve 100 mg in 1 ml of deionized water. TEMED is used neat.

6.3 Separating Gels - Calculating %T

Monomer Concentration (% T/2.67% C)	%T = x(%)
Acrylamides/Bis - 30% T	c ml
or for 40% T Stock*	or c' ml
Deionized water	d ml
1.5 M Tris-HCl, pH 8.8	2.5 ml
10% Ammonium Persulfate (make fresh daily)	50 µl
TEMED	5 µl
Total Monomer	10 ml (volume needed for two mini gels)

Determine (c ml) and (d ml) for 10 ml of total monomer:

c ml: Calculate the volume of 30% (or 40%) Acrylamide/Bis stock required for 10 ml of the desired total monomer concentration (x) with the following formula: For 30% stock:

c ml = (x%)(10 ml)/(30%) = (x)(0.333) ml

c'ml: *Similarly, for 40% stock solution:

c' ml = (x%)(10 ml)/(40%) = (x)(0.25) ml

d ml: Calculate the volume of water required at the desired total monomer concentration with the following formula:

d ml = (10 ml - 2.5 ml - 50 µl - 5 µl) - c ml = 7.445 ml - c ml

Important: One can prepare any desired volume of monomer solution by multiplying the 10 ml recipe by the appropriate multiplying factor.

6.4 Stacking Gel - 4% T (0.125 M Tris, pH 6.8)

Monomer Concentration (% T/2.67% C)	%T = x(%)
Acrylamides/Bis - 30% T	c ml
or for 40% T Stock*	or c' ml
Deionized water	d ml
0.5 M Tris-HCl, pH 6.8	2.5 ml
10% Ammonium Persulfate (make fresh daily)*	50 µl
TEMED	5 µl
Total Monomer	10 ml (volume needed for two mini gels)

* To make 10% APS, dissolve 100 mg in 1 ml of deionized water.

Section 7
Native-PAGE

7.1 Native PAGE Slab Gels

The discontinuous buffer system of Ornstein-Davis (Tris/chloride/glycine) should be the first non-denaturing gel system tried.[4] An advantage of discontinuous systems for dilute protein solution is the use of stacking gels to concentrate the sample. However, the stacking phenomena encountered in discontinuous systems can cause aggregation of some proteins and this can severely interfere with resolution.

The pH attained in the resolving gel of the Ornstein-Davis system approaches pH 9.5, which may be outside the range of stability for some proteins. Alternative discontinuous buffer systems derived for preparative work can be found in an article by Chrambach and Jovin.[5] The electrophoresis buffers described in this article span the pH range from pH 3 to pH 10.

If discontinuous systems cannot be used because of stacking-induced aggregation, a continuous buffer system will be required. In continuous systems the same buffer is used in the upper and lower electrode chambers and in the gel. McLellan describes various continuous buffer systems from pH 3.8–10.2 which can be tried.[2] See Section 7.4 for preparation of these continuous buffers.

7.2 Reagents for Discontinuous Native PAGE (Ornstein-Davis)

A. 30% Acrylamide Stock Solution

Acrylamide/Bis (30% T, 2.67% C)

146 g acrylamide (29.2 g/100 ml)

4 g N'N'-Bis-methylene-acrylamide (0.8 g/100 ml)

Dissolve in about 350 ml deionized water.

Make to 500 ml with distilled water. Filter and store at 4 °C in the dark.

Or substitute Bio-Rad's Preweighed Acrylamide/Bis 37.5:1 mixture or 40% Acrylamide/Bis Stock solution.

Caution: Acrylamide monomer is a neurotoxin. Avoid breathing acrylamide dust, do not pipet acrylamide solutions by mouth, and wear gloves when handling acrylamide powder or solutions containing it. For disposal of unused acrylamide, add bis-acrylamide (if none is present), induce polymerization, and discard the solidified gel.

B. Separating (resolving) Gel Buffer Stock

1.5 M Tris-HCl, pH 8.8

Dissolve 54.5 g Tris base in approximately 150 ml deionized water.

Adjust to pH 8.8 with 1 N HCl (Do not back titrate).

Make to 300 ml with deionized water and store at 4 °C.

C. Stacking Gel Buffer Stock

0.5 M Tris-HCl, pH 6.8

Dissolve 6 g Tris base in approximately 60 ml deionized water.

Adjust to pH 6.8 with 1 N HCl (Do not back titrate).

Make to 100 ml with deionized water and store at 4 °C.

D. Sample Buffer

Deionized water	4.8 ml
0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	2.0 ml
0.5% (w/v)bromophenol blue	0.2 ml
Total Volume	8.0 ml

E. 10x Electrode (Running) Buffer, pH 8.3 (Makes 1 Liter)

Tris base	30.3 g
Glycine	144.0 g

Dissolve in deionized water and adjust the final volume to 1,000 ml. **DO NOT** adjust pH with acid or base. To make 1 liter of 1x electrophoresis buffer (0.025 M Tris, 0.192 M glycine, pH 8.3) dilute 100 ml of 10x stock with 900 ml deionized water.

7.3 Prepare Ornstein-Davis Acrylamide Gels

Use the following table to prepare gels with %T ranging from 4%T to 10%T.

% T (acrylamide monomer)	Deionized H_2O (ml)	Gel buffer solution* (ml)	Acrylamide/Bis solution 30% stock (37.5:1) (ml)
4%	6.15	2.50	1.33
5%	5.80	2.50	1.67
6%	5.55	2.50	2.00
7%	5.15	2.50	2.33
8%	4.80	2.50	2.67
9%	4.47	2.50	3.00
10%	4.17	2.50	3.33

* Resolving Gel buffer - 1.5 M Tris-HCl, pH 8.8

* Stacking Gel buffer - 0.5 M Tris-HCl, pH 6.8

Catalysts	10% APS*	TEMED*
Resolving Gel	50 µl	5 µl
Stacking Gel	50 µl	10 µl

* Amounts are per 10 ml gel volume. To make 10% APS, dissolve 100 mg in 1 ml of deionized water. TEMED is used neat.

7.4 Reagents for Continuous Native-PAGE

In continuous systems, the same buffer is used in the electrode chambers and in the gels. Since stacking gels are not commonly employed, proteins migrate in bands at least as wide as the applied sample. Therefore, the sample volume must be kept at a minimum, *i.e.*, the sample should be at as high a concentration as possible. The mobilities of proteins in continuous systems are dictated primarily by pH rather than by sieving through the polyacrylamide gel.

For this reason, 6% polyacrylamide gels are recommended for most applications. For very large proteins 4% or 5% gels may be used.

Continuous and Elution Buffer Systems

McLellan describes various continuous buffer systems from pH 3.8 to pH 10.2 all with the same ionic strength.[2] Use the table below to prepare 5x continuous, non-denaturing buffers. Add both the acidic and basic components to 1 liter of water. Do not adjust pH with acid or base. If the final pH is outside the listed range, discard the buffer and remake it.

Buffer pH±0.1	Basic component and MW	5X solution g/L or ml/L	Acidic component and MW	5X solution g/L or ml/L
3.8	Beta-Alanine 89.09 mw	13.36 g/L	Lactic Acid 85% soln.	7.45 ml/L
4.4	Beta-Alanine 89.09 mw	35.64 g/L	Acetic Acid 17.4 M	11.5 ml/L
4.8	GABA 103.1 mw	41.24 g/L	Acetic Acid 17.4 M	5.75 g/L
6.1	Histidine 155.2 mw	23.28 g/L	MES 195.2 mw	29.5 g/L
6.6	Histidine 155.2 mw	19.4 g/L	MOPS 209.3 mw	31.4 g/L
7.4	Imidazole 68.08 mw	14.64 g/L	HEPES 238.33 mw	41.7 g/L
8.1	Tris 121.14 mw	19.38 g/L	EPPS 252.2 mw	37.85 g/L
8.7	Tris 121.14 mw	30.29 g/L	Boric Acid 61.83	7.73 g/L
9.4	Tris 121.14 mw	36.34 g/L	CAPS 221.3 mw	44.26 g/L
10.2	Ammonia 14.8 M	12.5 ml/L	CAPS 221.3 mw	22.13 g/L

To make 1 liter of 1X elution buffer, dilute 200 ml of 5X buffer with 800 ml deionized water. The final concentrations of buffer components will be:

Buffer pH	Basic Component	Acidic Component
3.8	30 mM Beta-Alanine	20 mM Lactic Acid
4.4	30 mM Beta-Alanine	40 mM Acetic Acid
4.8	80 mM GABA	20 mM Acetic Acid
6.1	30 mM Histidine	30 mM MES
6.6	25 mM Histidine	30 mM MOPS
7.4	43 mM Imidazole	35 mM HEPES
8.1	32 mM Tris	30 mM EPPS
8.7	50 mM Tris	25 mM Boric Acid
9.4	60 mM Tris	40 mM CAPS
10.2	37 mM Ammonia	20 mM CAPS

7.5 Prepare Continuous Gels (10 ml acrylamide monomer solution)

% T (acrylamide monomer)	Deionized H_2O (ml)	Gel buffer solution* (ml)	Acrylamide/Bis solution 30% stock (37.5:1) (ml)
4%	6.65	2.00	1.33
5%	6.30	2.00	1.67
6%	6.05	2.00	2.00

Catalysts	10% APS*	TEMED*
Resolving Gel	50 µl	5 µl
Stacking Gel	50 µl	10 µl

* Amounts are per 10 ml gel volume. To make 10% APS, dissolve 100 mg in 1 ml of deionized water. TEMED is used neat. Below pH 6, TEMED becomes less effective as a catalyst. Between pH 4 and pH 6, increasing the concentration of TEMED 5-fold to 10-fold will polymerize the gel.

7.6 Sample Preparation

Sample buffer for continuous native PAGE is a dilution of the electrophoresis buffer. The concentration of the sample buffer is generally 1/10 that of the running buffer. Glycerol is added to the sample buffer to a final concentration of 20%.

Section 8
Troubleshooting Guide

Problem	Cause	Solution
1. Incomplete elution of proteins from the gel.	a. Air bubble formation between the anode and cathode.	a. Refer to Section 3.2.
	b. Buffer or running conditions are not optimized.	b. Refer to Section 4.
2. No detectable proteins in collected fractions (complete elution).	a. Insufficient protein load.	a. Increase total protein loaded. Use silver stained SDS-PAGE gels to analyze individual fractions.
	b. Protein adhering to the cellophane membrane.	b. Reverse current for 10 seconds after the elution run. Decrease the run time.
3. Cross-contamination of fraction.	a. Elution chamber core not tightened down.	a. Tighten the hex screws in a crisscross pattern.
	b. Filter paper or cellophane membrane missing or placed in wrong order.	b. Refer to Section 3.2 (assembly).

Problem	Cause	Solution
4. Poorly defined fractionation of proteins.	a. Protein bands are not parallel to the elution chambers.	a. Carefully align the cutting template with the dye front and molecular markers of the gel when cutting.
5. Running conditions outside recommended range.	a. Buffer concentration or pH is incorrect.	a. Make fresh buffer.
6. Splattering of sample in vacuum harvester.	a. Vacuum is too strong.	a. Decrease vacuum.
7. Harvester is collecting uneven sample volumes.	a. Vacuum is too strong.	a. Decrease vacuum.

Section 9
Equipment and Accessories

Catalog Number	Product Description
165-1250	**Whole Gel Eluter**
165-1251	**Whole Gel Eluter with large harvester**
165-1255	**Mini Whole Gel Eluter**
165-1256	**Mini Whole Gel Eluter with Mini Harvester**
165-1260	**Large Harvester**
165-1261	**Mini Harvester**
Consumables	
165-1270	**Whole Gel Eluter Template**
165-1271	**Mini Whole Gel Eluter Template**
165-1275	**Cellophane for Whole Gel Eluter**, 25
165-1276	**Cellophane for Mini Whole Gel Eluter**, 25
165-1277	**Sealing Tabs for Whole Gel Eluter**, 50
165-1278	**Sealing tabs for Mini Whole Gel Eluter**, 50
165-1279	**Roller**, 1
165-1280	**Lower Chamber filter paper for Whole Gel Eluter**, 75
165-1281	**Upper Chamber filter paper for Whole Gel Eluter**, 50
165-1282	**Lower Chamber filter paper for Mini Whole Gel Eluter**, 50
165-1283	**Upper Chamber filter paper for Mini Whole Gel Eluter**, 50
223-9563	**DPTP, non-sterile, style M**, 500
223-9523	**DPTP, non-sterile, style D**, 500
223-9645	**Culture tubes, 12 x 75 mm**, 5 ml capacity, non-sterile, natural polypropylene, 2,000
223-9500	**Micro Test Tube, 1.5 ml,** polypropylene, natural, capless, 500

Catalog Number	Product Description
224-0100	**Micro Test Tube, 1.5 ml**, conical, with separate O-ring scewcaps, non-sterile, 500
165-1284	**Ruler, Whole Gel Eluter**
Accessories	
165-5050	**PowerPac 300**, 100/120 V
165-5051	**PowerPac 300**, 220/240 V
165-5054	**PowerPac 1000**, 100/120 V
165-5055	**PowerPac 1000**, 220/240 V
165-5004	**Vacuum Station**, 120 V, includes vacuum pump, vacuum, regulator, instructions
165-5005	**Vacuum Station**, 240 V
165-5006	**Vacuum Regulator**

Section 10
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